

AN IMMUNOLOGIC INVESTIGATION OF BISALBUMINEMIA:
A GENETICALLY TRANSMITTED SERUM PROTEIN ANOMALY

An abstract of a Thesis by
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November 1974
Drake University
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The problem. Bisalbuminemia was investigated in an Iowa family by testing for hematological and immunologic abnormalities to determine associations between bisalbuminemia and connective tissue (collagen) and/or autoimmune diseases. Physiochemical properties of the anomalous albumin fraction were compared to normal serum albumin.

Procedure. Cellulose acetate electrophoresis was used to determine the genotypes from which was established the genetic pedigree of the bisalbumin trait. Sera of family members was screened using the following tests: Complete blood count; sedimentation rate; hematocrit; antistreptolysin O titer; rheumatoid factor; LE preparation; VDRL; C-reactive protein and fluorescent anti-nuclear antibody analysis. Physiochemical characterization of the anomalous albumin fraction included an agar-gel diffusion study and measurement of the relative binding ability of normal and abnormal albumins using I^{125} -thyroxine.

Findings. Bisalbuminemia was found in seven of fourteen members of an Iowa family and was presumed to have been present in one deceased member of the family. The anomaly, transmitted as an autosomal codominant, was observed in the heterozygous state. The anomalous albumin fraction, albumin B, replaces one-half of the normal serum albumin and was found to be an albumin by agar-gel diffusion. Tests commonly associated with connective tissue and autoimmune diseases failed to show a relationship between the disease groups and bisalbuminemia. Addition of I^{125} -thyroxine to bisalbumin sera resulted in excess thyroxine binding to albumin B with greater affinity than to normal albumin.

Conclusions. The anomalous albumin B is assumed to be the result of a mutation of a gene responsible for the synthesis of normal serum albumin. The bisalbuminemia analyzed in this family study does not appear to be associated with the connective tissue and/or autoimmune diseases or any marked clinical abnormalities.

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A Thesis
Presented to
The School of Graduate Studies
Drake University

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by
Lee Stuart Vertuno

November 1974

1974
V617

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INTRODUCTION AND REVIEW OF THE LITERATURE

Bisalbuminemia, first reported by Scheurlen (1955), is an unusual serum protein anomaly characterized by the presence of two electrophoretically distinct albumins of approximately equal concentration in the same serum sample. Knedel (1957, 1958) observed the anomaly in eight members of two different families. Immunochemical studies indicated that both fractions in the albumin region, designated A_1 and A_2 by Knedel, were albumin and termed the condition "Double Albuminemia". Earle et al. (1958, 1959) reported the anomaly in 25 of 43 members of a single family designating the two albumin fractions A and B. Albumin A, corresponding to Knedel's A_1 , was proven to be normal albumin and albumin B, corresponding to Knedel's A_2 , was the abnormal albumin. Paralbuminemia was suggested by Earle et al. as preferable to bisalbuminemia, the latter term being appropriate for the heterozygous state only. Subsequently, several more families of European descent with slow moving albumin variants were described (Wuhrmann, 1959; Franglen et al., 1960; Miescher, 1960; Adner and Redfors, 1961; Cooke et al., 1961; Sarcione and Aungst, 1962; Robbins et al., 1963; Efremov and Braend, 1964; Sandor et al., 1965; Drachman et al., 1965; Braend et al., 1965; Ungari and Lopez, 1965; Adams, 1966; Weitkamp et al., 1966). An additional slow albumin variant found in Venezuelan Warao Indians was reported by Arends et al. (1969).

In addition, several fast moving albumin variants have been reported (Wieme, 1960; Tarnoky and Lestas, 1964; Melartin and Blumberg, 1966; Bell et al., 1967; Polesky and Rokala, 1967).

Melartin et al. (1967) found a variant termed Albumin Mexico which migrated between the normal albumin and the slow moving variant most frequently observed in persons of European origin.

Weitkamp et al. (1967) compared the electrophoretic mobility of 19 albumin variants obtained from unrelated families. These 19 variants were placed in five classes based upon their electrophoretic mobility. The slow moving variant from persons of European origin was designated as "very slow". The variants described by Sandor et al. (1965) and Melartin et al. (1967), presumed to have similar migration rates, were called "slow". The fast moving variants were divided into three classes. One called "fast" came from a British family described by Tarnoky and Lestas (1964). The variants described in North American Indians (Melartin and Blumberg, 1966; Bell et al., 1967; Polesky and Rokala, 1967) composed the "faster" class. The "very fast" category included the albumin described by Wieme (1960). It is evident, therefore, that in addition to normal albumin, there are at least five variants that give the appearance of bisalbuminemia (i.e., two albumin zones with similar staining intensity). An additional variant, which does not fit this definition, will be considered later.

Family studies on bisalbuminemia have shown the anomaly to be

transmitted genetically as an autosomal codominant characteristic and that the double albumin peak in the serum of an individual is a manifestation of the heterozygous state. Homozygotes have been reported by Melartin and Blumberg (1966) and Bell et al. (1967) in Indian subjects exhibiting the "faster" type of bisalbumin anomaly.

Blumberg et al. (1968) suggested the substitution of "alloalbuminemia" for "bisalbuminemia" to indicate albumin variation. Such a term could be applied to homozygosity where the term bisalbuminemia is not appropriate. Furthermore, it would indicate inherited variation in a manner analogous with the serologically determined allotypes (i.e., Gm, Inv and Ag).

As mentioned earlier, an additional variant albumin exists that does not fit the definition of bisalbuminemia. Laurell and Nilehn (1966) found five of 1550 unrelated orthopedic patients had an unusual albumin pattern on agarose gel electrophoresis which was inherited as an autosomal codominant trait. The albumin, normal in concentration, consisted of a single broad zone extending cathodically to the alpha 1 globulin region. The total albumin of the anomalous serum was separated into two components chromatographically, one consisting of normal albumin and the other of a more positively charged component with about one-third the normal albumin concentration. The unusual component reacted immunochemically as albumin. This finding proved that the anomaly under study was actually bisalbuminemia, although the

electrical charge difference was not sufficient to separate the normal and aberrant protein completely.

Previous studies have suggested but not proven any disease associated with bisalbuminemia. Probands have been described with diabetes mellitus (Scheurlen, 1955; Laurell and Nilehn, 1966); hepatitis (Knedel, 1957); congenital absence of one kidney and ureter with an undiagnosed disease of the remaining kidney (Earle et al., 1958, 1959); acrocyanosis with hyperkeratosis and joint deformity of fingers and toes (Franglen et al., 1960); Wegener's granulomatosis (Sarcione and Aungst, 1962); rheumatic fever (Robbins et al., 1963); carcinoma (Tarnoky and Lestas, 1964); mitral stenosis and nephrotic syndrome (Weitkamp et al., 1966); and epilepsy (Tschankov, 1971). Laurell and Nilehn (1966) indicated a possible association between bisalbuminemia and disease of the supporting tissue after finding five unrelated subjects with anomalous albumins on screening electrophoresis of 1550 sera from orthopedic patients but only one person in 3200 control subjects that showed the same serum anomaly. One patient was known to have systemic lupus erythematosus and the remaining five persons had long histories of bone and joint pain and disorders. These included a ruptured knee meniscus, recurrent dislocation of a shoulder and back pain. A familial study was conducted on one subject which resulted in finding nine of 24 family members with bisalbuminemia. All nine family members attested to long histories of joint and bone problems.

Blumberg et al. (1968) states that serum albumin is the major plasma protein involved in blood transport of cations, anions, dyes, drugs and various physiological substances. Several substances bind almost exclusively to albumin (i.e., bromophenol blue, fatty acid, bilirubin) and several other substances which bind to other serum proteins also bind to serum albumins. Examples of substances which bind to a serum protein in addition to albumin are thyroxine, which binds mainly to alpha globulin, and hemoglobin, which binds mainly to haptoglobin. Both of these will bind to albumin when they are present in sufficiently high concentration. Several studies have included a relative binding investigation between normal and anomalous albumin fractions. Franglen et al. (1960) observed no difference between the relative binding ability of normal and bisalbumin to *n*-tolyl- α -naphthylamine-8-sulphonic acid and sulfonphthalein dyes. Sarcione and Aungst (1962) found that I^{131} -thyroxine bound to albumin B rather than albumin A in bisalbumin sera but no difference was observed with bromophenol blue and Hg^{203} labeled Neohydrin. Blumberg et al. (1968) found that thyroxine and bromophenol blue bound more readily to the "fast" moving albumin variants than to albumin A. The action of certain drugs may be altered due to the differential binding between albumin variants and therefore may be of clinical significance.

The present investigation was the result of finding the bisalbumin trait in a 14-year-old male with rheumatoid arthritis. On

the basis of previous studies it was hypothesized that bisalbuminemia was related to a group of connective tissue disorders termed "collagen diseases" and/or autoimmune disorders. It was felt that this relationship might be elicited by studies on the serum of affected individuals and their families, testing for hematological and immunological abnormalities that are commonly associated with collagen and autoimmune diseases. Characterization of the chemical and physical properties of the anomalous albumin were conducted and compared with normal albumin.

MATERIALS AND METHODS

The index patient, a 14-year-old Caucasian male, was obtained through the generous cooperation of Jack J. Spevak, M.D. A medical history of the subject revealed that initial symptoms occurred at age 11 with a diagnosis of rheumatoid arthritis. Treatment was ongoing at the time of this study. The family members were contacted and blood samples were collected from the index patient and 13 additional family members.

Investigation of the hypothesis was conducted in three phases. Initially, a geneological study was made using electrophoretic analysis to establish the genetic pedigree of the bisalbumin trait. Family members were then screened, hematologically and immunochemically, using the following tests: Complete blood count, sedimentation rate, hematocrit, antistreptolysin O titer (ASO), rheumatoid factor, LE preparation;

VDRL, C-reactive protein analysis and fluorescent anti-nuclear antibody analysis. The final phase of study included a comparative immunodiffusion analysis of normal and anomalous albumin and measurement of the relative binding abilities of the two albumins incorporating the use of I^{125} -thyroxine. Detailed descriptions of the above procedures are contained in the following paragraphs.

Cellulose acetate electrophoresis in barbital buffer (pH=8.8, ionic strength=0.09) was carried out in the Gelman Seprater system using Sepraphore III strips and a Gelman Electrophoresis Chamber using Sepraphore III, 1" X 63/4" strips. The sera were separated using recommended voltage and time, stained in Ponceau S and cleared using 10% acetic acid in methanol. Protein fraction percentages were determined by scanning with the Gelman Digiscreen Densimeter.

Total proteins were determined on the Technicon Autoanalyzer employing a micro biuret reaction.

Immunochemical studies were carried out by the agar-gel diffusion procedure of Ouchterlony (1949) and modified by Sarcione and Aungst (1962). The serum proteins were separated by cellulose acetate electrophoresis using barbital buffer as described above. The protein areas of the air-dried unstained strips were located by cutting 0.4 cm strips from each edge of the cellulose acetate strips and staining with bromophenol blue. The unstained central segment was then aligned within the outer strips and the protein boundaries marked with pencil.

The protein areas were cut from the strips, rolled on a curved forceps and placed into wells of immunodiffusion plates (Nutritional Biochemicals Corp., Cleveland, Ohio). The center well was filled with goat anti-serum against human albumin; the peripheral wells with protein containing cellulose acetate strips were filled with 0.85 percent saline. The plates were incubated at room temperature (22°-25° C) and were photographed after 48 hours of incubation.

Measurement of the relative thyroxine binding abilities of the two albumins was carried out as described by Robbins and Rall (1955) and modified by Sarcione and Aungst (1962). Three different concentrations of 125 I-thyroxine (Dyroxin, Mallenkroft) were added to serum; the serum was incubated at room temperature for 15 minutes and then subjected to electrophoresis on cellulose acetate in barbital buffer (described above). Radioautographs of the air-dried unstained strips were prepared by placing the strips against Kodak X-ray film for 24-48 hours prior to developing. The position of the strips with respect to the film was marked by punching holes through both strips and film so that precise realignment of the subsequently stained strips and the developed film could be achieved.

Hematocrits, sedimentation rates (Winthrop) and differential blood cell counts were performed using standard methods of clinical analysis (Levinson and MacFate, 1969). Blood cell counts were performed on a Coulter Counter, Coulter Electronics, using recommended

procedures.

Reagents and test kits for the Antistreptolysin O titration, C-reactive protein analysis, rheumatoid arthritis factor analysis and VDRL test were obtained from Grand Island Biological Co., Grand Island, N.Y. Procedures used were supplied with materials.

Fluorescent anti-nuclear antibody analysis was performed at Mercy Hospital, Des Moines, Iowa.

RESULTS

Agar-gel diffusion. Agar-gel diffusion of proteins A and B against goat antiserum to human albumin (Fig. 1, top left) resulted in a continuous line of precipitation of equal density which did not cross throughout four days of observation. On the same plate, normal human serum resulted in a single precipitation line and the alpha 1 globulin fraction (P) was non-reactive with respect to the antiserum. The remaining two plates (Fig. 1, top right and bottom) illustrate the reactivity of the albumin fractions of twelve additional family members to goat anti-human albumin serum.

Geneological data. The sera of 14 members of the proband's family were subjected to electrophoresis on cellulose acetate (Fig. 2). The double albumin trait was found to be transmitted through the mother's family (Fig. 3). The father was not available for testing. In addition to the index, six other family members exhibited the trait; the mother, an uncle, the grandfather, a great aunt, a great uncle and the

great uncle's son.

Serum protein concentrations. Those individuals having bisalbuminemia showed no evidence for an association of the trait with any change in total protein, total albumin or protein fraction other than albumin (Table 1). The ratio of albumin B to albumin A was 1.0 with a range of 0.9 to 1.2. Serum protein concentrations of family members with normal albumin are given in Table 2.

Thyroxine binding. When small amounts of I^{125} -thyroxine (0.1 and 1.0 ug%) were added to sera, the radioactivity was found almost exclusively in the interalpha globulin zone of both normal and bisalbumin sera (Fig. 4). When the I^{125} -thyroxine concentration was increased to 10.0 ug%, the radioactivity was found in both the interalpha and the albumin zones as reported previously by Robbins and Rall (1955). However, in bisalbumin sera, although thyroxine binding in both the interalpha and albumin zones occurred, the radioactivity appears to be bound to albumin B more densely than to the expected albumin A when compared to the electrophoretic strips.

Hematological data. Individuals with bisalbuminemia exhibited no significant changes from normal blood values for the hematocrit, sedimentation rate and complete blood counts. Data was not listed for subject II-3 due to an insufficient quantity of blood sample for study. Hematocrit values were normal for all subjects tested. Sedimentation rates were within normal limits except for subjects II-9 and IV-1 in

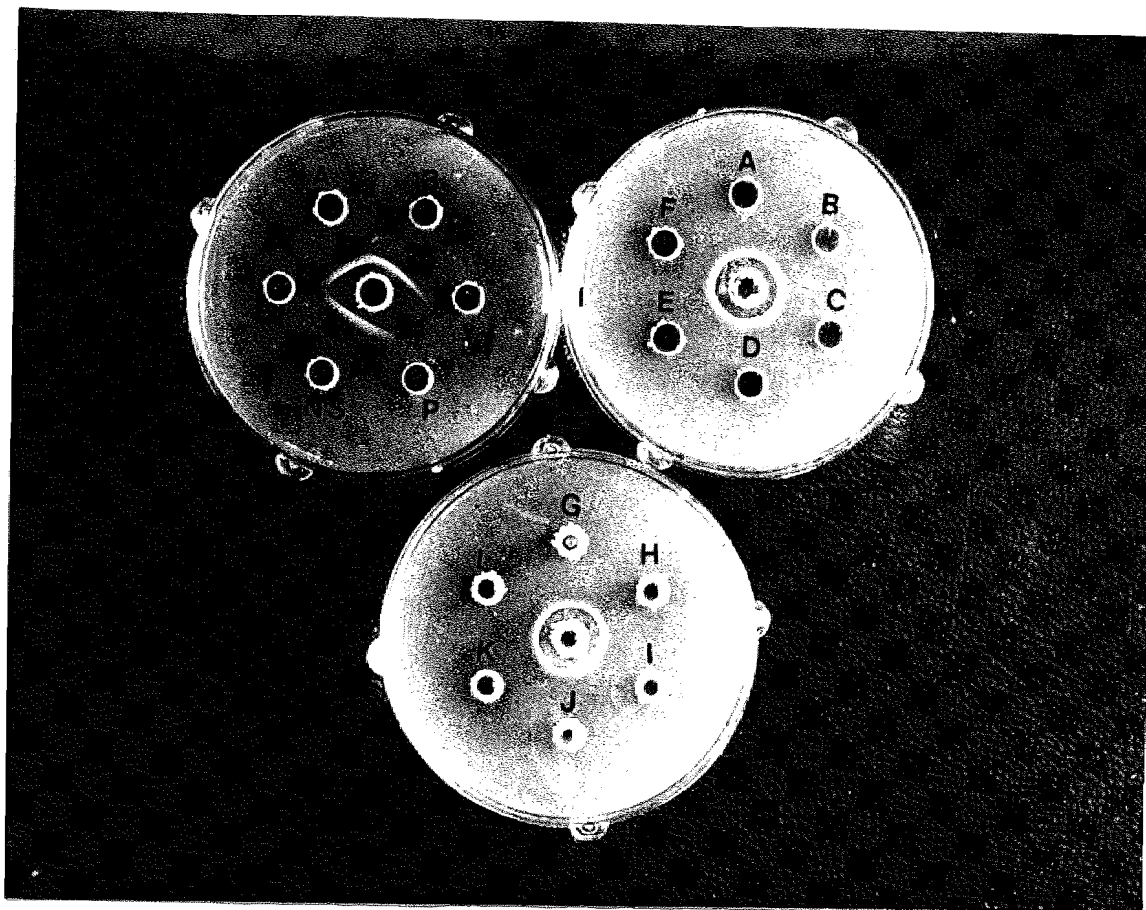


Figure 1. Agar gel diffusion precipitation reactions of goat anti-human albumin serum (center wells) against protein containing cellulose acetate strips (peripheral wells). Top left: Albumins A and B (subject IV-1), normal serum (NS) and normal alpha 1 globulin (P). Top right and bottom: Albumin fractions of twelve family members; A (II-1), B (II-2), C (II-5), D (II-7), E (II-9), F (III-1), G (III-3), H (III-4), I (III-5), J (III-7), K (IV-2), L (IV-4).

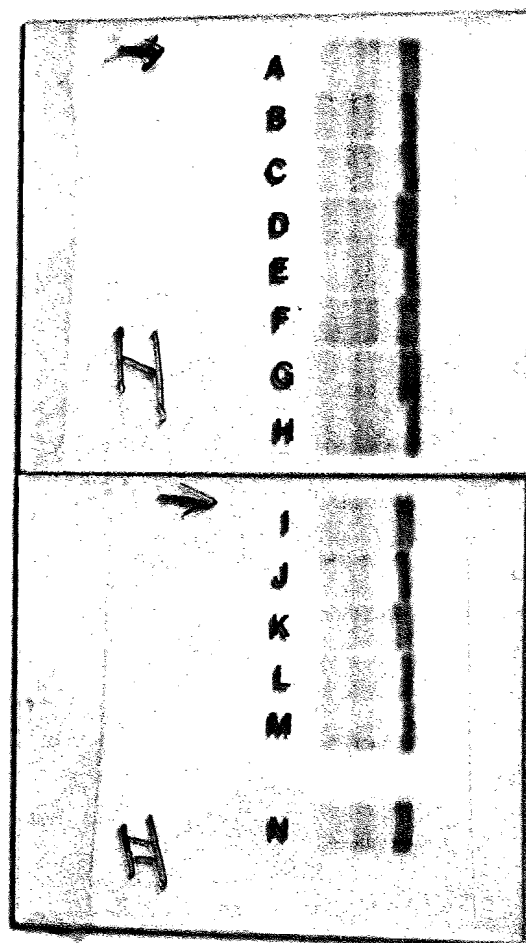
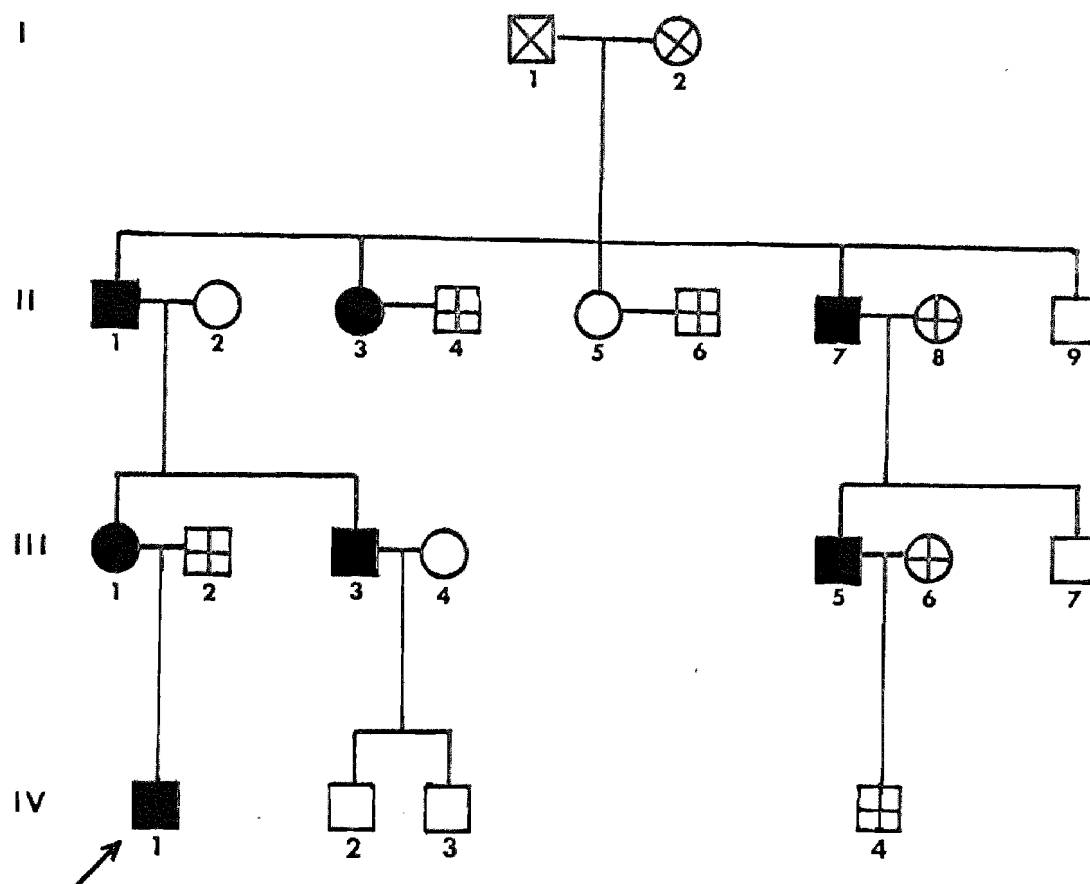


Figure 2. Cellulose Acetate Electrophoresis of sera from the family members who were tested.
A(II-1), (BII-2), C(II-5), D(II-7), E(II-9), F(III-1), G(III-3), H(III-4), I(III-5), J(III-7), K(IV-1), L(IV-2), M(IV-3), N(II-3).

Figure 3. Genetic pedigree of family with bisalbuminemia. Solid areas represent individuals having the anomaly. Arrow indicates index case with rheumatoid arthritis.



KEY

□ Male

⊠ Deceased

○ Female

⊞ Not Tested

Table 1. Protein Concentrations of Family Members with Double Albumin

Subject	Total Serum Protein	Serum Protein Fractions						Alb./Glob. Ratio	Albumin Ratios	
		percent total protein								
		gm%	Albumin		Alpha 1	Alpha 2	Beta		Gamma	
		A	B						A	B
II-1	6.6	33.7	29.8	3.9	9.9	9.4	13.3	1.72	1.0	0.9
II-3	7.1	29.0	31.0	4.5	10.3	10.3	14.9	1.50	1.0	1.1
II-7	5.3	34.2	30.6	3.5	8.5	10.0	13.0	1.86	1.0	0.9
III-1	8.2	26.7	26.6	2.7	17.1	13.2	13.7	1.14	1.0	1.0
III-3	7.1	29.4	34.6	4.7	9.9	10.4	10.9	1.78	1.0	1.2
III-5	7.2	36.3	32.1	3.6	6.7	6.1	15.1	2.17	1.0	0.9
IV-1*	6.9	30.2	32.2	3.4	10.1	10.1	14.0	1.66	1.0	1.1
Mean	6.9	31.4	31.0	3.7	10.4	9.9	13.6	1.69	1.0	1.0
±S.E.	0.3	1.3	1.0	0.3	1.2	0.8	0.5	0.13		
Normal Values	6.2	56.5		3.1	6.7	6.8	11.8	1.1		
	to 8.5	to 71.1		to 5.6	to 10.3	to 11.4	to 19.3	to 2.2		

*Index Patient

Table 2. Protein Concentrations of Family Members with Normal Albumin

Subject	Total Serum Protein	Serum Protein Fractions percent total protein					Alb./Glob. Ratio
		Albumin	Alpha 1	Alpha 2	Beta	Gamma	
	gm%						
II-2	8.3	52.1	4.2	13.6	14.0	16.1	1.09
II-5	7.4	58.1	4.5	13.5	13.1	10.08	1.39
II-9	6.9	57.0	5.4	13.0	13.0	11.6	1.32
III-4	6.9	51.8	8.9	13.1	13.7	12.5	1.07
III-7	7.7	62.5	3.7	10.6	8.8	14.4	1.67
IV-2	7.0	62.0	5.3	10.0	10.0	12.7	1.63
IV-3	7.3	60.7	4.3	10.4	10.4	14.2	1.54
Mean	7.4	57.7	5.1	12.0	11.8	13.2	1.39
±S.E.	0.2	1.7	0.7	0.6	0.8	0.7	0.09
Normal Values	6.2-8.5	56.5-71.1	3.1-5.6	6.7-10.3	6.8-11.4	11.8-19.3	1.1-2.2

which cases the values were above normal. Red blood cell and white blood cell counts were normal except for slight leukocytosis exhibited in subjects II-2, II-5 and III-1. Differential counts on the subjects were within normal limits except for the following. Slight lymphocytosis in subjects II-2 and IV-3 and slight eosinophilia in subject IV-2.

Immunochemical test data. Individuals with the bisalbumin trait showed no evidence for an association of the trait with any change in the LE prep, ASO titer, rheumatoid factor, VDRL, C-reactive protein or fluorescent anti-nuclear antibody analysis. The LE prep was negative for all family members. The ASO titer was high for three individuals, III-1, III-5 and IV-1. The rheumatoid factor test, VDRL and fluorescent anti-nuclear antibody tests were negative for all family members. The C-reactive protein test (quantitative) gave positive results for all subjects except IV-2 and IV-3 who were both negative. High titers were found in family members II-1, II-2, II-3, II-9, III-1 and III-3.

Clinical material. Case histories of the family members were obtained through personal interview or obtained from individual family physicians. Identification numbers refer to the pedigree (Fig. 3). Subjects' initials follow ID numbers.

I-1. E.D.; Deceased. Subject II-1 provided background of diabetes mellitis and emphysema. No history of bone or joint

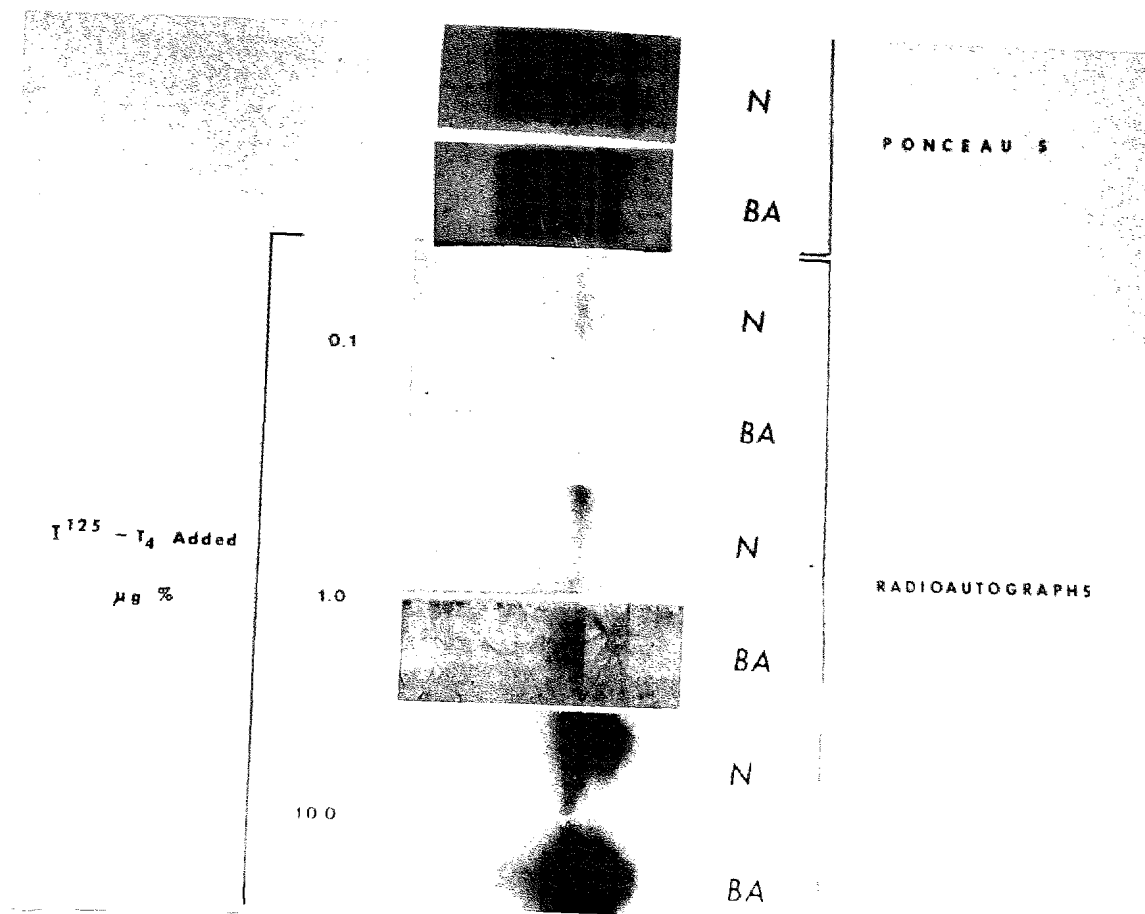


Figure 4. Binding of I^{125} -thyroxine (T_4) by normal (N) and bisalbumin (BA) sera. The topmost pair of strips show Ponceau S staining for localization of the protein fractions on cellulose acetate. The three pairs of radioautographs demonstrate the effects of increasing concentration of I^{125} - T_4 on its binding by serum components.

Table 3. Hematologic Data For Family Members

Subject	Hematocrit (percent)	Sed. Rate mm/hr.	Complete Blood Count						
			RBC	WBC	Differentials (percent)				
			$10^6/\text{mm}^3$	mm^3	Neut.	Eosin.	Baso.	Lymphs.	Mono.
II-1	47	1	5.691	5,997	64	0	1	28	7
II-2	41	4	5.118	11,525	43	1	2	49	5
II-3	---	---	---	---	---	---	---	---	---
II-5	44	5	5.922	12,519	66	1	1	28	4
II-7	44.5	4	5.429	7,729	66	2	0	24	8
II-9	44	10	5.180	7,500	70	2	1	18	9
III-1	48.5	3	4.792	13,950	76	2	1	16	5
III-3	44	0	5.000	8,478	65	5	0	21	9
III-4	36	1	5.932	7,842	68	3	2	24	4
III-5	46	7	5.234	5,264	63	1	1	33	2
III-7	52	0	5.345	6,389	50	3	5	37	5
IV-1*	40	12	5.483	7,687	60	2	1	33	5
IV-2	51	0	5.454	6,448	55	8	1	31	5
IV-3	46	0	5.616	6,808	41	5	1	44	9

*Index Patient

Table 4. Immunochemical Test Data for Family Members

Subject	LE Prep.	ASO Titer (Todd Units)	RAF	VDRL	C-Reactive Protein	F.A.N.A.
II-1	Negative	125	Negative	Non-Reactive	5	Negative
II-2	Negative	333	Negative	Non-Reactive	8	Negative
II-3	Negative	---	Negative	Non-Reactive	5	Negative
II-5	Negative	250	Negative	Non-Reactive	1	Negative
II-7	Negative	500	Negative	Non-Reactive	1	Negative
II-9	Negative	833	Negative	Non-Reactive	6	Negative
III-1	Negative	2500	Negative	Non-Reactive	8	Negative
III-3	Negative	250	Negative	Non-Reactive	5	Negative
III-4	Negative	333	Negative	Non-Reactive	2	Negative
III-5	Negative	2500	Negative	Non-Reactive	1	Negative
III-7	Negative	625	Negative	Non-Reactive	1	Negative
IV-1*	Negative	2500	Negative	Non-Reactive	1	Negative
IV-2	Negative	166	Negative	Non-Reactive	Negative	Negative
IV-3	Negative	250	Negative	Non-Reactive	Negative	Negative

*Index Patient

problems.

I-2. I.D.; Deceased. Subject II-1 provided information and background of tuberculosis. Bone or joint problems were denied.

II-1. O.D. is a 62-year-old Caucasian male with a 20 to 25 year history of arthritis affecting most joints. Subject had cancer of the lip at age 50 which was surgically excised. Individual appeared healthy but complained of chronic arthritis at time of interview. Subject exhibits bisalbuminemia.

II-2. P.D. is a 59-year-old Caucasian female with slight arthritis of ten year duration affecting the digits of both hands. Subject in apparent good health at time of interview.

II-3. T.D.M. is a 60-year-old healthy female. Bone and joint problems were denied and she has the bisalbumin trait.

II-5. M.C. is a 58-year-old woman with Hodgkin's disease. She has no history of joint or bone problems. Prednisone therapy in progress.

II-7. L.D. is a 58-year-old male and denies any pathology. He has bisalbuminemia.

II-9. E.D. is a 54-year-old man recovering from cerebral apoplexy. Presently under treatment for above, he denies history of bone and joint problems.

III-1. D.J.C.; a 36-year-old woman who has had, since the age of 12 years, diabetes mellitis treated with insulin. She has no renal complications and denies bone or joint symptoms. She exhibits

bisalbuminemia.

III-3. R.D.D. is a healthy 34-year-old man who has bisalbuminemia.

III-4. C.F. is a healthy 34-year-old female.

III-5. D.D.: No information available from subject but bisalbuminemia is present.

III-7. T.D.: No information from subject and he has normal serum.

IV-1. S.C.C. is the proband. He is a 14-year-old male with a history of rheumatoid arthritis since age 12 affecting the right wrist, right knee and left knee. Initial symptoms were evidenced two weeks following rubella immunization. Subject had rheumatic fever at age 9 years. Tonsils and adenoids were removed at age 4. Sinovectomy on right knee performed at age 13 years with good results. No acute pathology at time of interview. Cortisone therapy in progress.

IV-2. R.D.F. is a healthy 14-year-old schoolboy.

IV-3. R.S.F. is a healthy 12-year-old prepubertal male.

DISCUSSION

The abnormal protein observed in the index subject and six of his relatives by zone electrophoresis on cellulose acetate was identified as albumin using an agar-gel diffusion procedure. The modified Ouchterlony procedure with goat anti-human albumin serum revealed that both albumin A and B reacted in a manner indistinguishable from

normal human serum albumin. This observation indicates the identity or very close antigenic relation of albumins A and B to normal serum albumin. Electrophoresis revealed no additional abnormalities besides the double albumin in the sera tested. Control studies on normal human serum provided no evidence for the presence of a second albumin with a mobility between normal albumin and the alpha 1 globulin region.

The geneological data confirms the genetic transmission of the bisalbumin trait during three generations. Each individual with the anomaly is heterozygous for the characteristic, having both normal and abnormal albumin. Thus, affected persons probably received a normal gene from one parent and an anomalous gene from the other. All unaffected individuals of the family probably received a normal gene from each of their parents. The term "codominant" has been suggested because of the effect of each gene, the normal and abnormal seen in heterozygotes (Earle et al., 1959).

The presence of bisalbuminemia was not associated with any significant change in the total serum protein, total albumin or serum components other than albumin. The ratio of albumin B to albumin A in anomalous sera was 1:1 with a range of 0.9 to 1.2. Total serum albumin levels were normal. Earle et al. (1959) reported a similar 1:1 ratio for albumins A and B. Also reported by Earle et al. were ratios provided by Knedel (personal communication) of 3:2, 2:3, 2:1

and 1:2. These differences in relative proportion of albumins A and B were explained as a variation in the relative effectiveness of the mutant gene in different families. The albumin/globulin ratios were within normal limits for all subjects with the exception of II-2 and III-4 who had low values. Subject III-1, the proband's mother, had an elevated alpha-2-globulin value which is probably the result of an increased alpha-2-macroglobulin or pre-beta-lipoprotein level due to diabetes mellitus (Cawley, 1969). Although not statistically significant, the anomalous sera show some minor value changes in mean percentage of protein components alpha-1-globulin, alpha-2-globulin and total serum albumin. These minor percentage variations are not evident, however, when values are expressed in gram percent of protein in the various sera.

Study of the comparative binding ability of albumins A and B and normal serum albumin using I^{125} -thyroxine resulted in albumin B having a greater affinity to bind I^{125} -thyroxine than did albumin A (Cech, personal communication). Normal serum albumin bound excess thyroxine in control sera. Sarcione and Aungst (1962) in a binding study using I^{131} -thyroxine observed similar binding properties for albumins A and B and gave several explanations for the unexpected results. The initial speculation was that neither albumin A nor albumin B were identical to normal serum albumin. Modifications in the amino acid sequence and therefore changes in the number of charged

side chain groups occurred or such charge differences resulted in a difference in the folding of the polypeptide chains of the albumin molecule and resulted in binding property changes. A second possibility was that the amino acid sequence of albumin A is identical to normal serum albumin and that other factors influenced the thyroxine binding of albumin A. These factors might include the action of various buffer systems, such as veronal and barbitol, to change binding abilities by displacing binding sites. This explanation could be ruled out, however, due to the simultaneous use of normal serum and the observation that normal serum albumin always bound the added thyroxine. A third possibility was that the bisalbumin sera contained an inhibitor which prevents the binding of added thyroxine to albumin A. Blumberg et al. (1968) found similar binding properties for albumin B and albumin Reading (a fast type of bisalbuminemia). Thyroxine binding in albumin Naskapi, a fast type of bisalbuminemia, showed no difference in binding properties when compared with normal albumin.

Hematological and immunochemical tests failed to show an association of the bisalbumin trait with a disease entity. Hematological values were within normal limits with the exception of two subjects exhibiting slightly elevated values for white blood cell counts and one subject having a slight eosinophilia. Immunochemical tests were negative for the LE prep, rheumatoid factor, VDRL and fluorescent anti-nuclear antibody analysis. Three subjects showed high ASO titers and

all three exhibited the bisalbumin trait. However, this is probably a result of recent streptococcal infection when the blood samples were collected rather than an affect of the double albumin trait. The possibility does exist though, that persons with bisalbuminemia are more susceptible to infection with Streptococcus sp. A single titration is not sufficient for this evaluation to be made. The C-reactive protein test is indicative of an inflammatory response. The C-reactive protein appears simultaneously with the onset of inflammatory or malignant processes. Twelve family members exhibited positive titers with several showing high values. This would seem to indicate recent or acute pathology. Diagnosis would require further evaluation of the individuals. Retrospectively, the high titers may have been the result of an influenza epidemic immediately prior to collection of the blood samples.

The present investigation was unable to show that the collagen diseases or the autoimmune diseases are associated with the bisalbumin trait using the aforementioned hematological and immunologic tests. However, it did provide additional characterization of the chemical and physical properties of the anomalous albumin and further, provided an additional family having the bisalbumin trait for study.

SUMMARY AND CONCLUSIONS

A protein serum anomaly, bisalbuminemia, has been found on cellulose acetate electrophoresis of the serum proteins in 7 of 14

members of a family and is presumed to have been present in one deceased member of the family.

The anomaly has been observed in the heterozygous state and is transmitted as an autosomal codominant characteristic.

The anomalous albumin B is assumed to be the result of a mutation of a gene responsible for the synthesis of normal serum albumin.

Those individuals having bisalbuminemia showed no evidence for an association of the trait with any change in total protein, total albumin or protein fraction other than albumin.

The anomalous albumin fraction replaces one-half of the normal serum albumin and by agar-gel diffusion (Ouchterlony) analysis was found to be an albumin.

Addition of I^{125} -thyroxine to bisalbumin sera resulted in excess thyroxine binding to albumin B with greater affinity than to albumin A.

Hematological and immunochemical tests commonly used to diagnose connective tissue (collagen) and autoimmune diseases did not show a relationship between these disease groups and the bisalbuminemia cases in this study. Although this relationship was a major finding of this study, the possible association of these parameters in other patients cannot be ruled out.

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